

## Leptin: A metabolic signal affecting central regulation of reproduction in the pig

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### Abstract

The discovery of the obesity gene and its product, leptin, it is now possible to examine the relationship between body fat and the neuroendocrine axis. A minimum percentage of body fat may be linked to onset of puberty and weaning-to-estrus interval in the pig. Adipose tissue is no longer considered as only a depot to store excess energy in the form of fat. Recent findings demonstrate that numerous genes, i.e., relaxin, interleukins and other cytokines and biologically active substances such as leptin, insulin-like growth factor-I (IGF-I), IGF-II and Agouti protein are produced by porcine adipose tissue, which could have a profound effect on appetite and the reproductive axis. Hypothalamic neurons are transsynaptically connected to porcine adipose tissue and may regulate adipose tissue function. In the pig nutritional signals such as leptin are detected by the central nervous system (CNS) and translated by the neuroendocrine system into signals, which regulate appetite, hypothalamic gonadotropin-releasing hormone (GnRH) release and subsequent luteinizing hormone (LH) secretion. Furthermore, leptin directly affects LH secretion from the pituitary gland independent of CNS input. Changes in body weight or nutritional status are characterized by altered adipocyte function a reduction in adipose tissue leptin expression, serum leptin concentrations and a concurrent decrease in LH secretion. During pubertal development serum leptin levels, hypothalamic leptin receptor mRNA and estrogen-induced leptin gene expression in fat increased with age and adiposity in the pig and this occurred at the time of expected puberty. In the lactating sow serum and milk leptin concentrations were positively correlated with backfat thickness and level of dietary energy fed during gestation as well as feed consumption. Although, these results identify leptin as a putative signal that

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links metabolic status and neuroendocrine control of reproduction, other adipocyte protein products may play an important role in regulating the reproductive axis in the pig.

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## 1. Introduction

Discovery of leptin has improved our understanding of the relationship between adipose tissue and energy homeostasis [1]. Leptin produced by adipocytes, may act as a metabolic gate, which permits activation of the reproductive axis [2]. The hypothalamus appears to be a key site of action, since leptin receptors (OBR) are located within hypothalamic areas associated with control of appetite, reproduction and growth [3]. In the pig, nutritional perturbations delay onset of puberty, interfere with normal estrous cycles and alter LH secretion in the pig, for a review see Prunier and Quesnel [4]. It is hypothesized that mechanisms regulating energy balance are sensitive to metabolic signals generated by changes in oxidation of metabolic fuels and could account for positive correlations between body fat, fertility and endocrine function [5]. Thus, a relatively new concept of a “brain–pituitary–adipose–ovarian axis” has emerged.

## 2. Adipose tissue an endocrine organ

Adipose tissue plays a more dynamic role than previously thought in physiological mechanisms and whole-body homeostasis. Adipose tissue constitutes the largest amount of stored energy in the body [6]. Adipocytes have evolved into the primary storage site of triglycerides for the body’s long-term energy needs. Regulation of energy expenditure involves the balance among several factors such as, feeding behavior, adipose tissue mass and activation

Table 1

Microarray analysis of secreted factors expressed in neonatal pig adipose tissue and preadipocyte cell cultures: no previous published reports of pig adipose tissue expression

Growth related	Cytokines	Hormones	Others
Relaxin	Interferon (IFN- $\alpha$ 2, - $\gamma$ , - $\beta$ 1)	Follicle stimulating hormone $\beta$ -subunit (FSH- $\beta$ )	Agouti
Insulin-like factor-3 (INSL-3)	Interleukin (IL-1 $\beta$ , -4, -5, -8, -12, -15)	Luteinizing hormone $\beta$ -subunit (LH- $\beta$ )	Agouti-related protein (AGRP)
Brain derived neurotrophic factor (BDNF)	Tumor necrosis factor- $\alpha$ (TNF- $\alpha$ )	Corticotropin releasing hormone (CRH)	Plasminogen activator inhibitor-1 (PAI-1)
Growth differentiation factor-9 $\beta$ (GDF-9 $\beta$ )	Tumor growth factor- $\alpha$ (TGF- $\alpha$ )	Follistatin	Orsomucoid 1 (ORM1)
Nerve growth factor- $\beta$ (NGF- $\beta$ )	Small-inducible cytokine A2 (SCYA2)	Glycoprotein hormones alpha chain (CGA)	

Table 2

Proteomic analysis of preadipocyte cell culture conditioned media: protein hits with high confidence intervals (CI)

Protein	Accession no.	CI (%)	Species
HBEGF	Q01580	85, 60	<i>Sus scrofa</i>
BDNF	P14082	83, 82	<i>Sus scrofa</i>
IGFBP-5	Q28985	94, 90	<i>Sus scrofa</i>
Relaxin	P11185	92, 56	<i>Balaenoptera edini</i>
Interleukin-8	P36925	69	<i>Ovis aries</i>
Prolactin	Q28318	94	<i>Capra hircus</i>

HBEGF, heparin-binding epidermal-like growth factor; BDNF, brain-derived neurotrophic factor; IGFBP-5, insulin like growth factor binding protein-5.

of catabolic processes such as reproduction. Changes in body weight or nutritional status are characterized by alterations in serum concentrations of many hormones and growth factors that regulate adipocyte function and leptin secretion [1]. Recent microarray analysis by our laboratory revealed that 21 secreted protein genes were expressed 40-fold in neonatal porcine adipose tissue and porcine preadipocyte cultures (Table 1). Additionally, the agouti gene was detected by RT-PCR in pig adipose tissue. Proteomic analysis of adipose tissue and preadipocyte culture conditioned media identified several secreted proteins including relaxin and insulin-like growth factor binding protein (IGFBP-5; Table 2). Another secreted protein, plasminogen activator inhibitor-1 (PAI-1) was identified by ELISA in preadipocyte media. These studies demonstrate for the first time the expression of several major secreted proteins in pig adipose tissue, which may influence local and central metabolism and growth. Thus, these reports support the idea that adipose tissue functions as an endocrine organ.

### 3. Adipose depot innervation

Morphological studies revealed that adipose tissue is innervated by adrenergic nerve fibers [7]. Immunocytochemical data revealed that most of the subpopulations of the adrenergic OBR-immunoreactive neurons supplying fat tissue in the pig were positive for NPY and tyrosine hydroxylase immunoactivity [8]. Moreover, immunopositive neurons for OBR were located in the paraventricular nucleus, ventromedial nucleus, anterior hypothalamic area, preoptic area, arcuate nucleus and supraoptic nucleus [9]. These studies provide the first morphological data demonstrating that hypothalamic OBR containing neurons are transsynaptically connected to the perirenal fat depot. Neurons, which express OBR RNA are also located in hypothalamic areas involved in regulating LH [10] and GH [11] secretion. Therefore, the above evidence supports a direct link between hypothalamic neurons in the regulation of fat metabolism and reproduction.

### 4. Leptin a metabolic signal regulates LH and GH secretion

Circulating leptin concentrations are positively correlated with adiposity and positive energy balance in pigs and with long-term positive energy balance, leptin steadily increased

in plasma to reflect increased adipose tissue mass [12]. However, leptin levels did not increase dramatically following acute feeding. Conversely, leptin concentrations in plasma and adipose tissue rapidly and profoundly decreased as a result of food deprivation and negative energy balance, see reviews [1,13]. Clearly in pigs, metabolic state, specifically energy balance is a potent regulator of leptin secretion and gene expression [14–16]. Perhaps hormones and/or metabolites that are altered during feed restriction regulate fasting-induced changes in leptin gene expression. Insulin, glucose and IGF-I are significantly reduced in the fasted state in the gilt [5,16] and are potent regulators of leptin expression in the pig [1,14].

In the pig, pulsatile leptin secretion decreased by 24 h of a 28 h fast with no subsequent change in LH and GH secretion [16]. In contrast, short-term feed restriction for 7 days suppressed pulsatile LH secretion and serum leptin concentrations [15] in mature ovariectomized (OVX) gilts. To assess the effect of glucose on leptin secretion, treatment with 2-deoxy-D-glucose (2DG), a competitive inhibitor of glycolysis, increased serum GH concentrations and suppressed LH pulse frequency, while leptin secretion was unchanged [16]. Although, leptin may serve as a metabolic signal, which communicates metabolic status to the brain, the neuroendocrine response to acute energy deprivation may be dependent on other metabolic cues such as IGF-I, insulin and glucose during periods of acute undernutrition [17].

## 5. Gonadotropin secretion

The effects of leptin appear to be mediated through modulation of hypothalamic NPY expression [18]. In the pig, presence of biologically-active OBR (OB-RB) in the hypothalamus and pituitary [3] and the fact that leptin increased LH secretion from pig pituitary cells and GnRH release from hypothalamic tissue *in vitro* [19] suggests that leptin acts through the hypothalamic-pituitary axis. There is strong evidence from co-localization of leptin receptor mRNA with NPY gene expression that hypothalamic NPY is a potential target for leptin [8]. In the pig, central administration of NPY suppressed LH secretion [20] and stimulated feed intake and reversed the inhibitory action of leptin on feed intake [1]. However, NPY might not mediate the action of leptin, since leptin failed to effect NPY release from pig hypothalamic-preoptic area tissue fragments [19]. Furthermore, a recent report demonstrated that metabolic signals in part are communicated to GnRH neurons via the  $\gamma$ -aminobutyric acid neuronal pathway [21]. Therefore, the action of leptin at the central nervous system may be mediated via other neural peptides in addition to NPY such as proopiomelanocortin and agouti-related peptide [1].

## 6. Puberty and the “brain–pituitary–adipose tissue–ovarian axis”

Post-natally the various components of the brain–pituitary–ovarian axis of the pig are functional prior to normal onset of puberty [22]. However, there is little information regarding mechanisms within the brain that bring the various components of the reproductive axis together in a proper temporal relationship to initiate puberty. During pubertal development in the gilt, serum leptin concentrations increased [23] along with a concomitant increase in

LH secretion and serum estrogen levels [2]. It is hypothesized that estradiol modulates the hypothalamic-pituitary response to leptin and leptin gene expression during pubertal development. In support this idea, estradiol-induced leptin mRNA expression in adipose tissue occurred at the time of expected puberty in the OVX prepubertal gilt, but not in younger animals [23]. This was associated with greater LH secretion [2] and an age-dependent increase in hypothalamic OB-RB expression [24].

Intracerebroventricular administration of leptin failed to stimulate LH secretion in the intact prepubertal gilt [19], but did suppress feed intake [25]. However, leptin administration occurred during the period of heightened negative feedback action of estradiol on LH secretion [2], thereby blocking the action of leptin on the GnRH/LH pulse generator. Thus, the effect of leptin on LH secretion is associated with stage of sexual maturation in the pig. We suggest that leptin may serve as a permissive metabolic signal that may be necessary for activation of the reproductive axis but not as triggering signal for the onset of puberty.

## 7. Lactating sow

During lactation, feed intake of sows is often inadequate to meet nutrient requirements for maintenance and lactation. There is increasing evidence that nutrition, reduction in backfat and changes in metabolic state and associated changes in metabolite and metabolic hormones such as, insulin, IGF-I, GH and leptin, influence the reproductive axis in the sow [4].

In the primiparous and multiparous lactating sow, serum and milk leptin concentrations were positively correlated with backfat thickness and level of dietary energy fed during

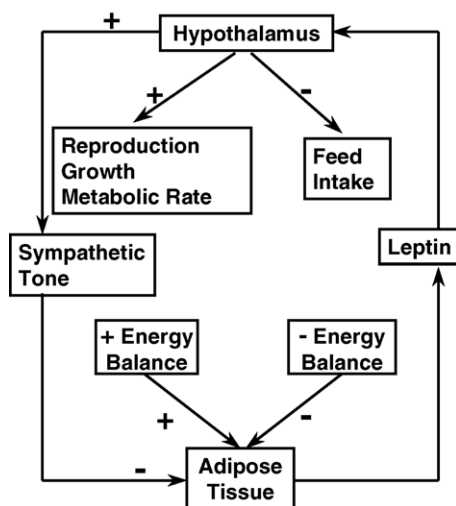


Fig. 1. Schematic illustration of leptin coordination of energy homeostasis and neuroendocrine function: leptin is secreted in response to changes in energy balance. Leptin acts on the hypothalamus to control food intake, reproduction and adipocyte function by suppressing expression of neuropeptide Y (NPY) and agouti-related peptide (AGRP) and upregulation of proopiomelanocortin (POMC) and increased sympathetic tone.

gestation, as well as feed consumption [26,27]. A positive correlation was observed among plasma insulin, leptin and LH concentrations in lactating sows fed ad libitum compared to feed restricted sows. Moreover, the weaning to estrus interval was greater in the feed restricted sows compared to controls [28]. These findings provide evidence that circulating leptin, LH concentrations and feed consumption during lactation are influenced by dietary energy intake during pregnancy or lactation in the sow. Thus, the role of leptin in modulating feed intake during lactation and post-weaning reproductive function remains to be determined.

## 8. Conclusion

Evidence was presented that supports the idea that leptin is more than a satiety signal. It serves as a metabolic signal exerting its effect by acting directly on the hypothalamus and pituitary gland to modulate the GnRH/LH secretory axis; feeding behavior and adipocyte function via the adrenergic innervation of fat depots (Fig. 1).

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